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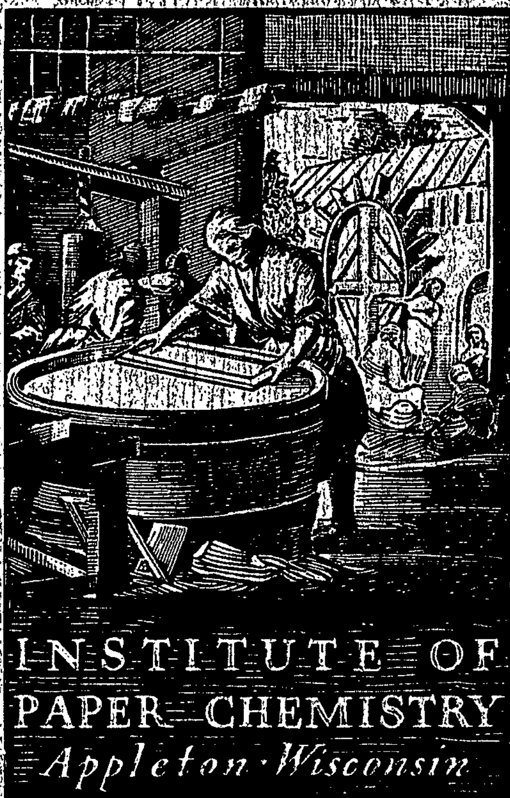

PRODUCTION AND INTENSIVE MANAGEMENT OF GENETICALLY IMPROVED ASPEN

Project 3537

Report One
A Progress Report
to
MEMBERS OF GROUP PROJECT 3537

March 15, 1986

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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Michigan Department of Natural Resources

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SUMMARY

The summer of 1985 marked the beginning of the expanded aspen project, moving it from primarily a seed production mode to one capitalizing on 30 years of research. Progress Report One describes the work being initiated as a result of the expanded project.

In anticipation of support for the expanded work with aspen, last year's (1984/85) crossing program included 18 full-sib crosses for use with a bioassay for hypoxylon screening. By anticipating this expanded work we were able to begin the hypoxylon screening immediately and not wait until this year's crossing program to produce part of the needed seedlots. Seven full-sib triploid hybrid crosses were also made and represented the major part of the crossing work. These crosses were used by cooperators for seedling production.

A mechanical sowing technique for aspen seedbeds was improved with the cooperation of the State of Wisconsin nursery at Hayward. The technique will be applicable to many current nursery operations.

Two demonstration plantings of hybrid aspen and one replicated field trial of quaking aspen crosses and one triploid hybrid cross were measured for growth and hypoxylon canker incidence. One of the demonstration plantings measured will be harvested and allowed to sprout back.

Work toward hypoxylon screening was begun utilizing a pathotoxin bioassay technique perfected by an Institute Ph.D. student. In that technique,

leaves from full-sib 16- to 18-week-old seedlings are challenged with a pathotoxin produced by *Hypoxylon mammatum*. Reaction to mammatoxin is determined by measuring the lesion diameter that develops. The validity of this technique for identifying hypoxylon resistant individuals will be determined in part through correlation with actual field data on hypoxylon incidence in full-sib progeny groups. If the correlation is high, the procedure could be used as a shortcut method for early screening of specific progeny groups and parent trees. It could also be used to identify clones with cellular-level resistance to mammatoxin. Preliminary results of the bioassay evaluations of progeny groups are presented.

The other important aspect of the program is the selection of outstanding clones for use in clonal forestry. Eleven selections were made this past year, including one natural triploid *P. tremuloides* selection made in 1956 that warranted inclusion. Seven of the selections were triploid hybrids and three were *P. canescens* x *P. grandidentata* hybrids exhibiting apparent resistance to the bronze leaf disease that seriously damages this type of hybrid. Growth and form of all the selections were exceptional. The selected clones will be propagated through conventional root sprout rooting techniques and outplanted in the Institute's Greenville arboretum to serve as the sources of material for large-scale production of planting stock.

Plans for 1986/87 include (1) continued work on the hypoxylon bioassay methods, (2) evaluation of field trials for hypoxylon incidence, (3) production of full-sib seedlots for use in hypoxylon screening, (4) hybrid aspen seed production, particularly triploid hybrid, for operational plantings, (5) propagation of seed orchard material, (6) selection of outstanding clones and propagating for inclusion in the Greenville arboretum, (7) measuring pertinent field trials for growth and survival information, (8) characterization of the wood quality of the selections, (9) an application to the Department of Energy for a grant to examine establishment and biomass production of hybrid aspen, and (10) cutting back one cooperator planting (PCA) and the Trial X sucker stand (second rotation) if arrangements can be made to do so.

INTRODUCTION

Quaking aspen is the most widely distributed hardwood species in North America. Aspen, both bigtooth and quaking, occupy 13,245,000 acres of forest land in the Lake States Region and are harvested in greater amounts than any other species. Roundwood harvest ranged from 42-51% of the total harvest for the period 1964-1984, with all other species combined making up the remaining 49-58%.

The short-term supply of aspen appears adequate, but an imbalance of age classes (too few acres in the younger age classes) suggests potential shortages may develop during the period 2000-2020. Shortages could persist if adequate harvesting and regeneration are not obtained during the years 1986 through 2000.

It is obvious that funding of aspen research is much below what it should be, considering its importance as a wood and fiber and wildlife resource. We were pleased with the response of the current cooperators to our proposal to bring the previous 30 years of aspen research out of mothballs and utilize the large collection of plant materials and field data available. The Institute has also contributed significant financial support in the area of hypoxylon research, which has allowed us to move rapidly into the objectives of the new aspen program. It is apparent, however, that for progress to continue at a rapid pace we will need to attract additional cooperators.

The prospect of a screening method to detect resistance to Hypoxylon mammatum, the most serious disease of aspen, killing an estimated 1-2% of the trees annually, is exciting and one of the major incentives for attempting to expand the aspen program. The other major incentive was our awareness of many outstanding hybrid aspen individuals in our field trials and outplantings, suitable for use in clonal forestry. Many of these individuals were from progeny groups with growth rates twice that of native aspen. These individuals, in turn, outgrew their siblings by up to 30%. The magnitude of gain that could be realized if these clones were propagated in large numbers was quite apparent. If the rapid growth rate of these clones can be further enhanced by improving survival over the rotation interval by screening for the most hypoxylon resistant clones, volume production could be in excess of 2 1/2 times that of native aspen.

Other hypoxylon related analyses are providing information on parental combinations producing progeny that are above average in hypoxylon resistance. It has only been because of the long-term nature of our work with aspen that we are able to have data to support our present work with hypoxylon resistance. We have found that progeny groups must be in the field for at least 10 years before hypoxylon infection begins and meaningful infection information does not occur until after 15 years.

We believe the support of the present core of cooperators reflects their understanding of the importance of aspen and the gains that can be achieved by beginning to utilize the fruits of 30 years of aspen research and being able to expand upon that research.

1985 CROSSING PROGRAM

The emphasis of the crossing program this past year was placed on two areas: the production of seed for large-scale outplantings and a series of small crosses for use in evaluation of resistance to the *Hypoxylon mammatum* fungus. The parent trees used in both areas have been progeny tested for up to 25 years. Progeny of this age are providing information on hypoxylon canker incidence, and it is because of these older trials and outplantings that we will be able to judge the usefulness of our bioassay method being employed as part of the expanded aspen program. They have and are providing information on wood quality, growth, and site requirements.

Twenty-nine full-sib crosses were produced and four open-pollinated *P. grandidentata* collections were made at the request of cooperators in West Germany and South Korea. Table 1 summarizes the 1985 crossing parentage. Table 2 summarizes the 1985 seed production.

HYPOXYLON SCREENING CROSSES

Eighteen crosses were made for use in a greenhouse bioassay for hypoxylon resistance. The parent trees used for this series of crosses have progeny in the field and are considered above average in performance. As can be noted in Table 1, five males were crossed with seven females, with each female being pollinated by more than one male. Hybrid crosses were made using three *P. tremula* females pollinated by the same series of *P. tremuloides* males used in the full-sib *P. tremuloides* crosses and they will be evaluated for the degree of resistance shown by the bioassay method. This will provide information on both the behavior of the males used with different females and the effect of interspecific hybridization on hypoxylon resistance.

›le 1. Summary of 1985 crosses and location of parent trees.

Cross Number[a]	Parents (Female	x	Male)
XT-1-85	T-53-60 (Fern, WI)	x	T-6-61 (Fern, WI)
XT-Ta-2-85	T-53-60 (Fern, WI)	x	Ta-10 (Ekebo, Sweden)
XT-3-85	T-5-61 (Ontonagon, MI)	x	T-46-60 (Ralph, MI)
XTa-T-4-85	Ta-6-68 (W. Germany)	x	T-46-60 (Ralph, MI)
XTa-T-5-85	Ta-6-68 (W. Germany)	x	T-20-60 (Alston, MI)
XTa-T-6-85	Ta-7-68 (W. Germany)	x	T-46-60 (Ralph, MI)
XTa-T-7-85	Ta-7-68 (W. Germany)	x	T-6-61 (Fern, WI)
XTa-8-85	Ta-6-68 (W. Germany)	x	Ta-1-68 (Wedesbuttel, Germany)
XTA-T-9-85	Ta-7-68 (W. Germany)	x	T-20-60 (Alston, MI)
XTa-T-10-85	Ta-8-68 (W. Germany)	x	T-44-60 (Ralph, MI)
XTa-T-11-85	Ta-8-68 (W. Germany)	x	T-46-60 (Ralph, MI)
XTa-T-12-85	Ta-8-68 (W. Germany)	x	T-6-61 (Fern, WI)
XTa-T-13-85	Ta-8-68 (W. Germany)	x	T-20-60 (Alston, MI)
XT-Ta-14-85	T-1-85 (Greenville, WI)	x	Ta-10 (Ekebo, Sweden)
XT-15-85	T-1-58 (Ontonagon, MI)	x	T-44-60 (Ralph, MI)
XT-16-85	T-1-58 (Ontonagon, MI)	x	T-46-60 (Ralph, MI)
XT-Ta-17-85	Clone 2 (Wausau, WI)	x	Ta-10 (Ekebo, Sweden)

Table 1 (Continued). Summary of 1985 crosses and location of parent trees.

Cross Number[a]	Parents (Female	x	Male)
XT-18-85	T-50-60 (Ralph, MI)	x	T-46-60 (Ralph, MI)
XT-19-85	T-50-60 (Ralph, MI)	x	T-6-61 (Fern, WI)
XT-Ta-20-85	T-50-60 (Ralph, MI)	x	Ta-10 (Ekebo, Sweden)
XT-Ta-21-85	T-12-58 (Clintonville, WI)	x	Ta-10 (Ekebo, Sweden)
XT-Ta-22-85	T-1-58 (Ontonagon, MI)	x	Ta-10 (Ekebo, Sweden)
XT-23-85	T-53-60 (Fence, WI)	x	T-46-60 (Ralph, MI)
XT-24-85	T-1-85 (Greenville, WI)	x	T-46-60 (Ralph, MI)
XG-25-85	G-10-66 (Dexterville, WI)	x	G-1-57 (Wausau, WI)
XT-Ta-26-85	T-30 Control (Eagle River, WI)	x	Ta-10 (Ekebo, Sweden)
XTa-27-85	Ta-6-68 (W. Germany)	x	XTa-3-68, S-1 (Greenville, WI)
XT-28-85	T-1-58 (Ontonagon, MI)	x	T-2-56 (Bruce Crossing, MI)
XT-29-85	T-1-58 (Ontonagon, MI)	x	T-2-56 (Bruce Crossing, MI)
XG-0-30-85	G-4-85 (Navarino, WI)	x	Wind
XG-0-31-85	G-5-85 (Navarino, WI)	x	Wind
XG-0-32-85	G-1-85 (Bldr. Junction, WI)	x	Wind
XG-0-33-85	G-3-85 (Laona, WI)	x	Wind

[a]See Appendix for description of crossing code.

Table 2. Summary of 1985 seed production.

Cross[a]	No. of Catkins Pollinated	Number of Seeds	Seeds/ Catkin Pollinated	Germination, %	Purpose
XT-1-85	7	31	4	--	Hypoxylon screening
XT-Ta-2-85	1,003	53,580	53	--	Seedling production
XT-3-85	4	1,238	310	76	Hypoxylon screening
XTa-T-4-85	16	8,637	540	95	Hypoxylon screening
XTa-T-5-85	15	9,553	636	--	Hypoxylon screening
XTa-T-6-85	14	6,421	459	93	Hypoxylon screening
XTa-T-7-85	16	8,353	522	--	Hypoxylon screening
XTa-8-85	16	24	2	--	Hypoxylon screening
XTa-T-9-85	10	6,212	621	--	Hypoxylon screening
XTa-T-10-85	7	109	15	--	Hypoxylon screening
XTa-T-11-85	12	577	48	--	Hypoxylon screening
XTa-T-12-85	11	1,321	120	--	Hypoxylon screening
XTa-T-13-85	10	1,856	186	--	Hypoxylon screening
XT-Ta-14-85	237	38,825	164	89	Seedling production
XT-15-85	17	854	50	89	Hypoxylon screening
XT-16-85	13	1,950	150	92	Hypoxylon screening
XT-Ta-17-85	172	7,205	42	--	Seedling production
XT-18-85	43	6,400	149	88	Hypoxylon screening
XT-19-85	12	4,686	390	89	Hypoxylon screening
XT-Ta-20-85	255	43,341	170	92	Seedling production
XT-Ta-21-85	134	19,252	144	--	Seedling production
XT-Ta-22-85	280	39,796	142	93	Seedling production

Table 2 (Continued). Summary of 1985 seed production.

Cross[a]	No. of Catkins Pollinated	Number of Seeds	Seeds/ Catkin Pollinated	Germination, %	Purpose
XT-23-85	12	1,305	109	94	Hypoxylon screening
XT-24-85	--	--	--	--	Tissue Culture Group
XG-25-85	34	5,758	169	--	South Korean Cooperator
XT-Ta-26-85	300	1,995	7	--	Seedling production
XTa-27-85	25	3,328	133	--	Hypoxylon screening
XT-28-85	--	--	--	--	Tissue Culture Group
XT-29-85	--	--	--	--	Tissue Culture Group
XG-0-30-85	830	997,988	1,202	--	West German and South Korean Cooperators
XG-0-31-85	398	50,019	125	--	West German and South Korean Cooperators
XG-0-32-85	530	74,189	140	--	West German and South Korean Cooperators
XG-0-33-85	727	82,422	113	--	West German and South Korean Cooperators

[a]See Appendix for description of crossing code.

Additional information on the work with the bioassay method can be found in the section of this report entitled Hypoxylon Screening.

TRIPLOID HYBRID SEED PRODUCTION

The second phase of the 1985 crossing program was the large-scale production of triploid hybrid seed for use in producing stock for cooperator outplantings. A total of 202,000 triploid hybrid seeds were produced last year, one of the lower production rates since the project was placed in a "maintenance mode." All of the seed was utilized by the Project 3537 cooperators and the State of Wisconsin nursery at Hayward. Although not a formal member, the State of Wisconsin requested at the time our aspen work was being reduced that they be able to purchase seed. The discussion with the aspen cooperators reached the consensus that seed could be purchased by the State of Wisconsin after the seed requests of the cooperators were satisfied. The cooperators were also to have an opportunity to purchase a portion of the seedlings produced by the state.

The *P. tremula* tetraploid male clone, Ta-10, has flowered consistently for the past three years in the Greenville arboretum. The flower stimulation techniques of bark inversion and strangulation have been quite successful and have freed us from having to rely on the original clone in Sweden for pollen. Pollen production has been good enough to allow us to store a portion for the next crossing season as a hedge against Ta-10 not flowering in the Greenville arboretum. Fortunately this has not occurred and we have mixed each year's stored pollen with fresh pollen as an extender and then stored a portion of the current season's pollen.

Packaging Corporation of America established a triploid hybrid seed orchard while a member of the aspen project. Although they withdrew from the program several years ago and elected to work with black poplars, their triploid seed orchard was maintained. PCA has since dropped the black poplar program and has sold the nursery and seed orchard area. We were fortunate to reach an agreement with the farmer that now owns the orchard. We are leasing it on an annual basis and intend to make extensive branch collections from it and reduce collections from our arboretum. The orchard is flowering and a collection will be made for this year's crossing program.

There are also two clones of tetraploid _P. tremuloides_ males that were produced through a pollen screening technique employed in 1968 and 1972. Both of these clones are now flowering, and crosses resulting from earlier small quantities of pollen collected from these clones have been outplanted. The availability of _P. tremuloides_ tetraploids allows the production of hybrids using _P. tremula_ females, the reciprocal of the current triploids being produced. Although this type of triploid has not been tested for any length of time, it has been planted in a replicated trial of the U.S. Forest Service on its Harshaw farm near Rhinelander, Wisconsin. The trial is designed to evaluate irrigation and fertilization treatments on black poplar materials. At the time the U.S. Forest Service requested stock from us for an outplanting, we did not realize they wanted our best triploid material. We looked at it as an opportunity to evaluate an unknown material in a replicated trial. After three years in the trial the material is doing very well. Figure 1 illustrates a number of triploid individuals. The form and appearance of this new triploid hybrid is very similar to the first type of triploid hybrid. Although growth and survival information were not available for this report we hope to have the data for next year's report.

Figure 1. Two male tetraploid P. tremuloides produced during earlier aspen project work are now flowering, and hybrid crosses with female P. tremula have been made to evaluate their progeny. The individuals shown are in the third growing season on a USFS replicated trial near Rhinelander, Wisconsin. Growth and survival are very good, and overall appearance is similar to the triploid hybrids produced from a male P. tremula tetraploid.

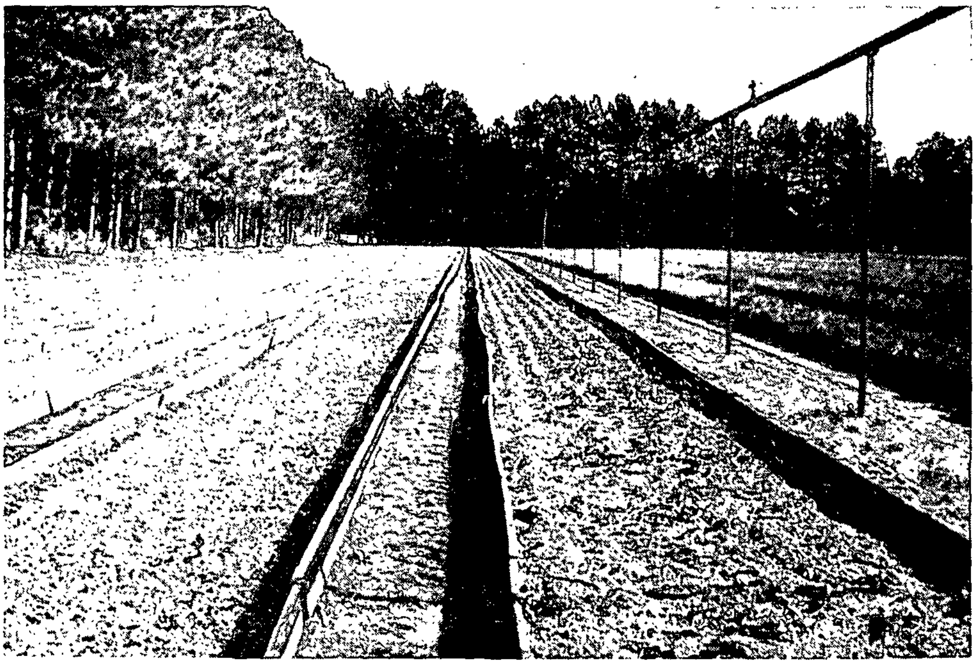
SEEDBED METHODS - MECHANICAL SOWING

The availability of progeny tested, superior parent trees, and the increased seed production from these parent trees has led to a need for mechanized hybrid aspen seedling production. Over the years we have modified seedbed techniques for growing hybrid aspen. There have been recent developments that appear to have improved planting stock production.

Dean Einspahr visited with our European aspen cooperators during a recent trip to Switzerland. One of the cooperators presented its seedbed method of hybrid aspen production. The main difference from our method was the European cooperator's ability to sow mechanically, using a machine it developed. The most interesting aspect was its use of a cereal about the same size and weight as aspen seed for a carrier. Discussions with a state nurseryman led to our use of a Love seeder that has as one of its features the ability to sow small volume seedlots. By using a similar cereal carrier, several 400-foot seedbeds were sown this past summer with excellent results (Fig. 2).

Seedbed preparation was not greatly changed from the past; beds were fumigated, aerated, lightly raked just prior to sowing, then sowed on the surface and watered in by an overhead irrigation system. Sideboards, hardware cloth (1/2-inch), and 50% shade cloth were then placed on the beds. The critical factor in hybrid aspen seedling production, however, remains the watering regime; the surface of the seedbeds cannot dry during the first 2-3 weeks, but neither can the top 2-3 inches of soil remain constantly saturated.

The seedbeds that resulted from this year's mechanical sowing are shown in Fig. 2, two months after sowing. Because of the difficulty in obtaining a plantable 1-0 seedling in the northern portions of the states of Wisconsin, Michigan, and Minnesota we are examining the possibility of a midsummer sowing and growing the seedlings to a height of about two inches before fall frosts. The seedlings remain in beds over winter and grow through the following year. If height growth appears to be excessive, the beds can be undercut. In the past, ılone was not sufficient to retard growth, but it is believed that the ıt ability to seed aspen in rows within seedbeds will permit lateral ıg as well as undercutting and root wrenching.



;ure 2. A method of mechanically sowing hybrid aspen seed is being developed in cooperation with the Wisconsin Department of Natural Resources Nursery at Hayward, Wisconsin. A Love seeder, capable of sowing small seedlots, is used to sow hybrid aspen seed mixed with a cereal carrier. Germination and bed density were good. Being able to plant seed in rows will allow lateral root pruning as well as undercutting to control stock size.

FIELD TRIAL AND DEMONSTRATION PLANTING EVALUATION

IPC 54 BEAR LAKE - PACKAGING CORPORATION OF AMERICA

A small demonstration planting of 6950 P. canescens x P. grandidentata and P. canescens x P. tremuloides hybrids was established in May, 1970 on Packaging Corporation lands east of Bear Lake, Michigan in Lower Michigan. The site was a former hay field with a soil texture of sand to sandy loam classified as Ubly sandy loam with 2-6 percent slopes. Six different crosses were planted in four blocks with the majority of the stock being canescens x bigtooth hybrids.

Survival was variable at the end of the fifth year ranging from 30-85%. Average height growth ranged from 20 feet for one of the canescens x tremuloides hybrid crosses to 12 feet for one of the canescens x bigtooth hybrid crosses. The bronze leaf disease that has seriously affected P. grandidentata hybrids was noted in this planting in 1976 and the progression of the problem was evaluated for several years. The incidence and severity of the disease caused mortality in the bigtooth hybrid materials and was present in the tremuloides hybrid material but did not cause mortality or lessen growth in those hybrids.

Observations in 1984 and again in 1985 led to a decision to cut the planting this coming year. The high mortality rate within the bigtooth crosses and the continuing incidence of the leaf disease problem in the survivors have reduced the usefulness of this part of the planting. Three clonal selections were made that have good growth, form, and no evidence of the leaf disease problem. These selections will be propagated and planted out with other apparently resistant materials to confirm their resistance. After cutting, the area will be allowed to form a sucker stand and the positions of the resistant clones marked and the development of suckers from those selections will be watched.

The canescens x tremuloides hybrid, although exhibiting moderate symptoms of the disease during its initial occurrence, have not had mortality associated with the disease. Although survival is low (29%) and is related to early establishment problems, growth of the survivors has been very good, averaging 50.6 feet and 7.2 inches dbh at 16 years. The block of this hybrid is about 4.5 acres in size and has about 126 trees/acre. Many stems are over 60 feet, and the largest tree measured was 70 feet and 12.3 inches dbh. The suckering that develops after cutting will be evaluated, and it should thoroughly occupy the area.

Based on the long-term resistance shown by the *P. canescens* x *P. tremuloides* hybrid to the leaf disease in this area of heavy incidence, we intend to make a canescens x tremuloides cross this year and begin once again to outplant small numbers of this type of hybrid for further evaluation.

IPC 62 WAUSAUKEE - AMERICAN CAN COMPANY

The American Can planting near Wausaukee, Wisconsin was planted on April 23, 1972 on a small, old-field site. The soil texture was sand with a ground cover of quackgrass and sweet fern. The forest cover surrounding this old abandoned homestead is bigtooth aspen, red maple, poor quality white and red oak, and scattered jack and red pine. Three types of hybrid aspen and five full-sib *P. tremuloides* crosses were planted at a tight spacing of three feet within rows and nine feet between rows.

When searching our crossing and planting records for parentage combinations that could be evaluated as part of a Ph.D. student's work with hypoxylon canker, several desired crosses occurred in this planting. It was apparent from the records that the planting had not been visited since the second year after planting. Deer pressure was recorded as heavy the first year and slight the second year. No other information was recorded.

The planting was visited in October, 1984 for possible hypoxylon evaluation as part of student work. The survival of one _P. tremuloides_ cross was very good and was rated for hypoxylon incidence. The material of most interest was a _P. tremula_ x _P. tremuloides_ hybrid. At 14 years of age the hybrid averaged 44.7 feet in height and 5.0 inches dbh (Fig. 3). There were numerous

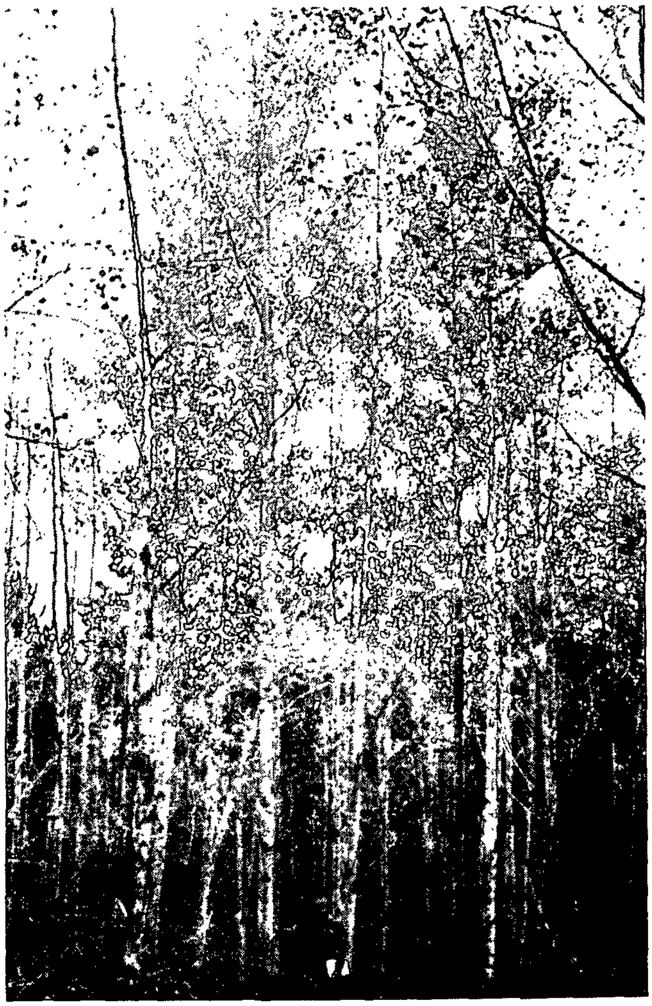


Figure 3. A 14-year-old diploid _P. tremula_ x _P. tremuloides_ planting near Wausaukee, Wisconsin on American Can Company land. Average height was 44.7 feet and 5.0 inches DBH. The potential of this type of hybrid is becoming more apparent as plantings begin to reach older ages. Many stems in this planting were over 50 feet with the tallest tree having a height of 61 feet and a 7.1-inch DBH.

stems over 50 feet with the largest tree having a height of 61 feet and a diameter b.h. of 7.1 inches. Survival was 36% and can probably be attributed to deer pressure and tight spacing, although no observations of early establishment problems were taken. However, the potential of this type of hybrid was demonstrated and additional crosses and outplantings are planned. This particular cross was made in Germany, using pollen from one of our selected males (T-44-60) on one of their selected females. We have since propagated that female and it is flowering in our Greenville arboretum.

TRIAL XXIV RIPCO TEST AREA - IPC

Trial XXIV was measured for growth and survival and scored for hypoxylon incidence this past fall. The trial is located in Oneida County, Wisconsin, south of Eagle River. The test area compartment on which Trial XXIV is planted is now owned by Wausau Papers, and permission to continue to use it and maintain it has been received.

The objective of Trial XXIV when it was planted in 1965 was to evaluate crosses of selected _P. tremuloides_ progeny from 1956 and 1957 crosses, utilizing a bigtooth cross as a control. A repeat cross, XT-Ta-6-64, was also included to further look at a diploid _P. tremuloides_ x tetraploid _P. tremula_ material (triploid hybrid).

Table 3 presents 5, 10, 15, and 21 year growth data for the six materials in Trial XXIV. The three crosses with the best growth, XT-2-64, XT-5-64, and XT-Ta-6-64 share a common female parent, T-1-58, a selection from the Porcupine Mountains in Upper Michigan. Unfortunately, two of those crosses, XT-2-64 and XT-Ta-6-64, also have poor hypoxylon ratings, but the third cross, XT-5-64, has the best hypoxylon rating in the trial. Although our analysis of hypoxylon occurrence is just beginning, we are noting considerable differences in resistance, depending upon the parental combination. The low incidence of hypoxylon in XT-5-64 is a good example of that difference in resistance.

Table 3. Trial XXIV - Ripco Test Area - 5, 10, 15, 21 year growth and survival.

	5-Year		10-Year		
Material[a]	Av. Ht., feet	Survival, %	Av. Ht., feet	Av. dbh, inches	Survival, %
XT-2-64	7.6	94	25.2	2.7	88
XT-3-64	6.4	91	20.6	2.1	80
XT-4-64	7.2	96	20.8	2.0	90
XT-5-64	9.0	92	25.2	2.5	88
XT-Ta-6-64	9.9	100	32.6	3.9	92
XG-14-64	3.2	56	9.0	0.5	33

	15-Year			21-Year		
Material	Av. Ht., feet	Av. dbh, inches	Survival, %	Av. Ht., feet	Av. dbh, inches	Survival, %
XT-2-64	40.3	4.5	83	54.8^{wx}	6.5^{x}	61^{wx}
XT-3-64	34.9	3.8	67	49.4^{xy}	5.6^{xyz}	46^{xy}
XT-4-64	33.1	3.6	86	46.2^{yz}	5.2^{yz}	74^{w}
XT-5-64	39.8	4.2	91	54.8^{wx}	5.9^{xy}	77^{w}
XT-Ta-6-64	48.2	6.3	77	61.5^{w}	9.0^{w}	42^{y}
XG-14-64	22.5	2.2	20	40.4^{z}	4.5^{z}	17^{z}

[a] See Appendix for description of crossing code.

^{wxyz} Duncan's Multiple Range Test was calculated when "F" test values for treatments were significant. Values followed by a common superscript letter are not significantly different.

The continuing decline in survival of the triploid hybrid XT-Ta-6-64, a cross between T-1-58 and Ta-10, reflects both the increased incidence of hypoxylon from age 15 to age 21 and an unusual occurrence of a black bark disease that produced mortality. A U.S. Forest Service pathologist is examining the problem, and it is being considered localized and unusual, but not serious. Of greater concern was the excessive hypoxylon incidence within this particular cross, a cross with outstanding growth in a number of trials and demonstration plantings. Further work will add to the evaluation of hypoxylon incidence in this parental combination in plantings on other sites. If the evaluation of this cross on other sites continues to indicate a high rate of hypoxylon incidence, we will avoid using it.

These kinds of results further demonstrate the need for testing a material for its anticipated rotation age. Many of the parent trees used in the aspen program have progeny in the field on a variety of sites and climatic conditions. The data being collected from the older plantings (20 years +) are providing both growth information and information on the impact of disease. Not only must we be aware of parental combinations that produce rapid-growing progeny, we need to also consider the potential disease ramifications of a particular cross.

Trial XXIV is providing an opportunity to evaluate growth and disease within five progeny groups. The very low incidence of hypoxylon in one of those progeny groups provides the opportunity for second generation selection and breeding for improved hypoxylon resistance. If the validity of the bioassay technique described elsewhere in this report can be demonstrated, it will provide a method by which we can go into progeny groups demonstrating good hypoxylon resistance and rapidly evaluate potential parent trees.

HYPOXYLON SCREENING

INTRODUCTION

Hypoxylon canker (Hypoxylon mammatum Wahl. Miller), as we have discussed in previous reports, is the most serious forest management problem influencing the future use of aspen and aspen hybrids. Over the years, there has been no shortage of research involving all aspects of the "hypoxylon problem." There are a number of positive indicators that suggest the hypoxylon canker problem can be solved using a combination of tree breeding, plant pathology, and tissue culture techniques. We feel the Institute is in the unusual position of having several hundred 15- to 25-year-old full-sib crosses in field plantings that have been evaluated periodically since establishment, of having evaluated parent trees, and of having tissue culture and pathotoxin bioassay capabilities. A breakthrough in solving the problem appears possible in the next two or three years if a reasonable level of research can be maintained. Most of the research presently underway involves determining the validity and reliability of the pathotoxin leaf bioassay procedure used in evaluating seedlings and rooted root sprouts.

The approaches we are presently using include

1. Evaluating the "hypoxylon status" of parent trees and full-sib progeny groups by remeasuring existing field tests
2. Determining the reliability and validity of the leaf bioassay method by a) making repeated measurements of the same clone, and b) measuring the bioassay response of rooted root sprouts from trees (seedling origin) that are disease free after 25 years and comparing the results with the bioassay response of trees of the same cross that are still alive but have hypoxylon

3. Using the leaf bioassay procedure to a) evaluate highly valued parent trees and b) determine the relative mammatoxin resistance of seedling populations, primarily crosses where long-term field records on "hypoxylon status" exist
4. Using the leaf bioassay procedure to evaluate the mammatoxin resistance status of quaking aspen and hybrid aspen clones selected for outstanding growth

Research is underway on the above approaches, and although none of the work on any one of the studies has been completed, progress has been good and a summary of progress follows.

FIELD DATA

A total of four replicated field trials and three cooperator plantings have been evaluated over the past two years. This work provided information on 48 full-sib crosses and allowed us to rate these experimental crosses after 20 to 25 years in the field as to their relative susceptibility to hypoxylon canker. Although we have reevaluated less than half of the total number of crosses we plan to consider, there have been a number of interesting results and patterns that seem to be developing. One disturbing fact is that after 25 years many of our full-sib crosses have 60% or greater infection, i.e., 60% of the trees from a full-sib cross either have died from or presently have hypoxylon canker.

Another interesting result is the greatly differing pattern of development of hypoxylon infection within a cross over time, suggesting more than one mechanism of resistance exists. Some crosses quickly develop a high level of infection (many trees are infected), and then very few trees in the cross become infected after year 15. Most crosses, however, have relatively few trees infected prior to year 10, and then the percentage of trees infected increases gradually until only a few trees remain alive at age 25. We also have two or three crosses that have very low levels of infection until age 20, and then some condition (hail, insects, temperature, etc.) apparently triggers a major increase in the level of infection. The most encouraging information emerging from the field data and the full-sib evaluations is that there are specific "female x male" combinations that produce crosses with better than average hypoxylon resistance. The resistance mechanism(s) involved remain to be elucidated.

HYPOXYLON SELECTION AND CROSSING PROGRAM

The selection and crossing research is designed to determine the validity and reliability of the leaf bioassay procedure (described in the section that follows) and at the same time generate hypoxylon resistant individuals that can be used in future breeding and clonal forestry research. The crosses used in the full-sib evaluation work include crosses made in 1984 and 1985 along with seed from stored crosses made earlier. All, for the most part, are repeats of earlier crosses for which we have field evaluation data extending back for 20 to 25 years. The crosses vary in the degree of resistance to hypoxylon (based on field data), and the objective is to evaluate these crosses using 25-30 seedlings and determine what kind of correlation exists between the bioassay information based on leaves from 16- to 18-week-old seedlings and the field evaluation. Should the correlation be high, the procedure could be used as a shortcut method for early screening of specific progeny groups and in the evaluation of parent trees.

The selection work also has a twofold purpose: first to test the validity of the leaf bioassay method and second to generate clones with cellular-level esistance to the mammatoxin that causes injury to aspen. During 1985, 40 elections were made and included 1) disease-free hybrids of exceptional form ınd growth rate, 2) disease-free quaking aspen of good form and growth, and 3) ıypoxylon infected aspen from the same full-sib crosses used in 2. Rooted root sprouts from these selections are to be used to help determine the validity and reliability of the bioassay method.

BIOASSAY METHOD

The bioassay method being used was perfected by Steve Wann as part of his thesis program. The mammatoxin being used in the leaf bioassay was produced by the liquid culture method as described by Schipper[1] and by Griffin[2]. Briefly, this involves growing isolates of Hypoxylon mammatum in liquid culture for 18 days at 28°C, removing mycelia by filtration, and using the filtrate as a source of mammatoxin. The filtrate is treated with methanol, the resulting precipitate is discarded, and after evaporating to dryness, the acidified residue is extracted using ethyl acetate followed by n-butanol. These two extracts can be used separately, but in these tests the two fractions were combined and used as a single mammatoxin source.

The leaf puncture bioassay used was the method described by Bruck and Manion[3], Stermer[4], and Griffin[2]. Mature leaves from 16- to 18-week-old greenhouse-grown seedlings and/or rooted root sprouts were excised at the point of attachment to the stem and placed in vials of water. Small holes were made in the leaf blade with a minutin insect pin and a 3 μL drop of mammatoxin placed over the hole. Following incubation in a humidified chamber at 28°C for 48 hours, response to mammatoxin was measured as lesion diameter (Fig. 4). Replication was obtained by using three tests (lesion measurements) per leaf and three leaves per plant.

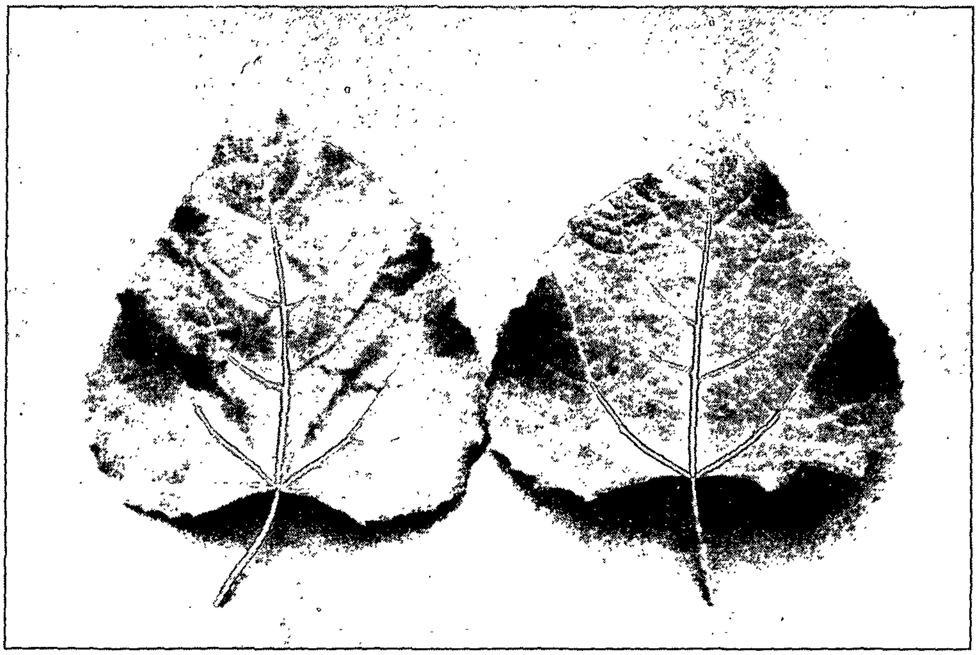


Figure 4. The response of two different P. tremuloides seedlings to Hypoxylon mammatum mammatoxin in a leaf puncture bioassay technique is illustrated. The leaf on the left has a susceptible reaction as measured by the black lesion diameter. The leaf on the right demonstrates a nonreaction to mammatoxin. It is hoped that by characterizing full-sib seedling populations and correlating the bioassay results with actual long-term field data on the incidence of hypoxylon, an early screening technique can be developed.

BIOASSAY RESULTS

Progeny Group Evaluations

The greatest amount of hypoxylon work this past year involved the evaluation of seedling populations. Approximately 385 seedlings from 16 full-sib crosses were evaluated using the leaf bioassay procedure. Most of the results obtained are summarized in Table 4. Although the work is not complete, there are a number of interesting trends developing. First, and of most interest, are the relatively large number of trees found to be what we consider as highly resistant to mammatoxin at a cellular level (lesions < 2 mm in diameter). These are trees that are part of a specific seedling population in which there is a good chance they are not only resistant to mammatoxin but are also resistant to the disease. This latter point remains to be verified. Second, and also of great interest, is the large variation that exists between crosses in the number of highly and moderately resistant individuals. This variation is not unexpected.

Table 4. Results of leaf bioassay evaluations of progeny groups.

Cross No.[a]	Parent female	x male	No. Seedlings Evaluated	No. Seedlings by Lesion Class <2 mm	2-5 mm	>5 mm	Mammatoxin Response Rating[b]
XT-6-80	T-20-56	T-46-60	25	5	5	15	4.5
XT-8-80	T-25-56	T-46-60	19	3	6	10	4.4
XT-4-82	T-53-60	T-44-60	25	8	12	5	3.2
XT-3-83	XT-22-56 S-2	T-44-60	25	7	13	5	3.3
XT-2-84	T-24-60	T-13-58	25	5	18	2	3.2
XT-16-84	T-5-61	T-44-60	25	3	9	13	4.5
XT-17-84	T-50-60	T-44-60	25	4	7	14	4.5
XT-18-84	T-5-61	T-20-60	25	3	7	15	4.7
XT-19-84	T-50-60	T-20-60	25	1	0	24	5.8
XT-3-85	T-5-61	T-46-60	29	0	12	17	5.0
XTA-T-4-85	Ta-6-68	T-46-60	29	15	11	3	2.5
XTa-T-6-85	Ta-7-68	T-46-60	19	4	8	7	3.9
XT-18-85	T-50-60	T-46-60	28	2	7	19	5.0
XT-19-85	T-50-60	T-6-61	17	0	5	12	5.3
XT-23-85	T-53-60	T-46-60	24	7	9	8	3.6

[a]See Appendix for description of crossing code.
[b]Equivalent to the weighted average lesion diameter.

The third pattern of variation, and one of some concern, is the lack of a consistent pattern of resistance that allows us to quickly select specific male or female parent trees that in all crosses produce an acceptable percentage of trees that are "highly resistant." More data will be required to establish the type of genetic relationship that exists and what approach should be used in selecting future parent trees.

Evaluation of Selected Clones

To examine the reliability of the bioassay method and to evaluate a number of clones of interest, rooted root sprouts were produced for the five clones listed in Table 5. When the greenhouse-grown potted root sprouts were 16 to 28 weeks old, leaves were collected and evaluated using the standard leaf bioassay procedure. As the results indicate, the method is very reproducible, and a closer examination of the data suggests a clone could be evaluated using as few as four ramets per clone. Another point of interest is that the clones selected from Populus tremula x P. tremuloides hybrid crosses show a very high level of cellular resistance. These are three clones selected for their exceptional form and growth rate from northern Wisconsin field trials.

Table 5. Clonal leaf bioassay results.

Clone[a]	Sex	No. Ramets Tested	Av. Lesion Dia., mm	Standard Deviation
T-50-60	Female	10	6.9	0.49
T-20-56	Female	13	4.8	0.72
XT-Ta-6-64 S-1	--	10	0.9	0.10
XT-Ta-10-58 S-5	--	10	0.9	0.09
XT-Ta-10-69 S-1	--	10	1.4	0.21

[a]See Appendix for description of crossing code.

Comparisons Between Diseased and Disease Free Clones

Root collections were made this past fall in a 21-year-old full-sib field planting, and the production of rooted root sprouts is presently underway. These collections consisted of three trees from selected crosses that were free of hypoxylon and three trees from the same cross that were alive but presently had hypoxylon. Leaf bioassays will be run and comparison made of mammatoxin resistance information. This work is preliminary in nature and will be expanded in the coming year to include additional crosses and selected trees. The number of comparisons will be dependent on the level of available funding.

CLONAL SELECTIONS

The expanded aspen program was initiated because of the potential gains from improved hypoxylon canker resistance and the availability of exceptional hybrid aspen individuals. The clonal forestry approach utilizing exceptional individuals is being recognized as a method to achieve considerable gains in several areas ranging from wood quality to site adaptability.

Hybrid aspen plantings over 15 years of age will provide the material from which clonal selections will be made. The impetus for initiating the clonal aspect of the project was the frequent observation of exceptional individuals in various plantings and the realization that if a method could be developed to economically propagate these clones, the gain in volume production and wood quality could be as much as 15-20% greater than a good hybrid aspen seedling plantation. Use of tissue culture as a propagation method also appears feasible.[5]

1985 SELECTIONS

Several of the selections made this past year had been noted in previous years, but because of the structure of the aspen program at the time we were not able to utilize them. Their locations were recorded in the event that future work would allow their use, and several were collected this past fall. Table 6 lists the first eleven selections. Clone T-2-56 is a natural triploid *P. tremuloides* that was selected in 1956 as part of a polyploid study. It has been propagated and included in several trials over the years, and because of its growth rate and high rate of survival is one of possibly two natural triploids that warrant inclusion in a clonal forestry program.

Table 6. Hybrid aspen clonal selections.

Clone Identification[a]	Location[b]	Age	Height, feet	DBH, inches
XT-Ta-10-58, S-5	X	26	74	11.4
XT-Ta-14-58, S-22	X	20	72	9.7
XT-Ta-14-58, S-23	X	26	76	12.9
XT-Ta-6-64, S-1	XXIV	20	75	11.7
XCa-G-37-67, S-1	IPC 54	16	58	11.0
XCa-G-38-67, S-1	IPC 54	16	58	8.9
XCa-G-38-67, S-2	IPC 54	16	54	9.0
XT-Ta-10-69, S-1	ST III	15	59	9.2
XT-Ta-10-69, S-2	ST III	15	60	7.6
T-2-56	--[c]	42	82	13.4
XT-Ta-#1[d]	X	21	76	11.9

[a]See Appendix for description of crossing code.
[b]Test trial or cooperator planting number.
[c]A 1956 natural triploid selection from Bruce Crossing, Michigan.
[d]A sucker origin selection in Trial X on the Ripco Test Area from one of two triploid crosses in the trial.

Three of the first selections were P. canescens x P. grandidentata hybrids from Packaging Corporation's planting, IPC 54, described earlier in this report. One of the major criteria for their selection was their apparent resistance to the as yet unknown leaf disease that produced extreme mortality in this and other plantings. The other criteria were growth rate and form. The selections exhibited no disease symptoms and had healthy, live crowns that stood out in stark contrast to the infected stand. At age 16, the selections ranged from 54-58 feet in height and 8.9 to 11.0 inches dbh (Fig. 5). The same type of hybrid has been planted in other areas and there are individuals showing resistance also. The canescens x bigtooth hybrid was produced for use on dry sites and was showing good promise until the leaf disease problem. The apparently resistant selections will be further tested for their resistance and their suitability on dry sites.

Figure 5. Two P. canescens x P. grandidentata hybrid selections from a 16-year-old planting on Packaging Corporation of America land near Bear Lake, Michigan. Three selections were made based on their apparent resistance to the bronze leaf disease that has caused a high rate of mortality in this type of cross, and for their growth rate and form. The clone shown on the left is 58 feet in height and 11.0 inches DBH. The clone on the right is 58 feet in height and 8.9 inches DBH.

The remaining seven selections are P. tremuloides x P. tremula triploid hybrids from three replicated trials. All seven selections have excellent form and growth rate (Fig. 6). Although the wood quality of each selection has not yet been determined, this type of hybrid aspen ranks among the best in specific gravity and has the best fiber length. The triploid hybrids will be used primarily on low- to medium-quality hardwood sites.

Figure 6. A 21-year-old triploid hybrid aspen clonal selection from Trial X on the Ripco Test Area. The selection is a sucker that resulted when a portion of the trial was cut back at age five for a sprouting study. The individual shown is 76 feet in height and 11.9 inches DBH. Selections of this quality are being made and propagated for use in clonal forestry.

Future selection will emphasize triploid hybrids, but additional clones will also be selected from _P. canescens_ hybrids and from diploid tremuloides x tremula and tremula x tremuloides hybrids. The Wausaukee planting described in this report and several other areas with diploid _P. tremula_ and _P. tremuloides_ hybrids are demonstrating that these materials have considerable promise. We will be making selections from appropriate plantings and will be producing greater amounts of seed of this type of hybrid.

PROPAGATION METHODS

After the clonal selections have been made they will be propagated and placed in the Greenville arboretum, where the breeding material and other clonal material accumulated over the course of the aspen project have been preserved. The propagation technique is one that has been developed and used in the aspen project for over 20 years. It involves collecting roots from the clonal selections and forcing them to produce shoots in the greenhouse, which are then excised and rooted (Fig. 7). Rooting success varies by clone, but on the average is expected to be over 80%. The first ramets (members of a clone) propagated will be placed in a root arboretum and will provide roots for the large-scale production of clonal planting stock.

Unlike the black poplars, which are easily propagated from stem cuttings, the aspens are difficult and in some instances almost impossible to root from stem cuttings. The _P. alba_ x _P. grandidentata_ and _P. canescens_ x _P. grandidentata_ hybrids can be rooted from stem cuttings, although there is variation from clone to clone. The _P. tremuloides_ x _P. tremula_ hybrids are very difficult to root from cuttings. However, there are approaches to improving rooting success, and a small greenhouse trial will be initiated to examine them. Although more labor intensive, the use of root sprouts for the production of planting stock is a feasible method.

Figure 7. The majority of the selections made for use in clonal forestry will be propagated from root sprouts. The one foot length of root illustrated demonstrates the number of sprouts that can occur. The sprouts are excised, rooted, and placed in a nursery lineout and are field plantable after one growing season.

Another propagation method that is becoming more widespread in its application, particularly in agronomic and ornamental crops, is tissue culture. The work by Steve Wann in his Ph.D. Thesis with hypoxylon utilized tissue culture methods for both clonal plantlet production and hypoxylon screening techniques. It became apparent that he was able to produce very large numbers of shoots from a small source of tissue.[5,6] It still required excision and rooting steps, but because of his ability to produce large numbers of individuals we believe the technique warrants a trial to determine the practicality of using this type of propagation method in the aspen program.

PLANS FOR 1986-87

The progress during the first year of the expanded project was very good and will provide the basis for much of the work planned for this coming year. The emphasis of our work will continue to be on hypoxylon resistance, particularly the acquisition of data from both experimental laboratory methods and actual incidence of hypoxylon in field trials and plantings of full-sib seed sources. Part of the crossing program will produce full-sib seedlots that have corresponding older age field plantings with the same parentage. The seedlots and field data will be used for hypoxylon screening.

Hybrid aspen seed production for use in nursery and container seedling production will continue on as large a scale as flower material permits. Triploid hybrid seed will again be the major emphasis of the crossing program, but two or three other types of hybrids will also be produced. The *P. canescens* x *P. tremuloides* hybrid will be reexamined for use on dry sites now that its resistance to the bronze leaf disease appears to be good.

Seed orchard material for triploid hybrid seed production will be propagated for those cooperators desiring to establish an orchard.

The selection of outstanding clones from as many outplantings and trials as possible will continue. After selection they will be propagated and placed in the Greenville arboretum. A characterization of each selection's wood properties will also begin.

Field trials that have materials for use in providing information for the present aspen program will be measured. A number of trials have not been measured since the program was placed in a maintenance mode. If measured, they could provide data pertinent to the present program.

An application for a Department of Energy biomass production grant has been made in cooperation with Consolidated Papers, Inc. If approved, the work will provide additional information on establishment methods, site selection, and early growth rate directly applicable to the aspen program.

The hybrid aspen planting of Packaging Corporation at Bear Lake, Michigan, will be harvested by PCA, and we will evaluate volume production and suckering of *P. canescens* x *P. tremuloides* hybrids. We will also mark the location of bronze leaf disease resistant individuals and follow the development of suckers arising from their root systems and from infected individuals to evaluate the incidence of bronze leaf disease on suckers.

The 21-year-old triploid hybrid aspen sucker stand in Trial X on the Ripco Test Area is another stand that should be cut. Most stems are merchantable, and the development and evaluation of the second rotation sucker stand would be useful information. However, the area is now owned by Wausau Papers and an agreement to allow us to cut must first be reached. It is hoped this can be arranged within the next year.

ACKNOWLEDGMENTS

We wish to acknowledge the very valuable technical assistance provided by Dr. Steven Wann. Without his help the routine leaf bioassay procedure we are using would not be a reality. It is also important that we acknowledge the funding provided by the Institute through its "funded" exploratory research program. These funds were used to further perfect the procedures used and make the preliminary measurements reported. We also wish to acknowledge the assistance of Bob Arvey and Egon Humenberger for their work with producing the full-sib crosses, field trial measurements, bioassay determinations, and greenhouse and nursery care. Our thanks also go to Betty Dorman and the Institute's Editorial and Publications Department for the typing and polishing of this report.

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THE INSTITUTE OF PAPER CHEMISTRY

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APPENDIX

A code system was devised early in the aspen program to handle the numbering of individual parent trees and crosses. It was necessary to incorporate into the system the ability to identify the species of selected parent trees, the type of cross (parentage) when used for crosses, and the year the trees were selected or the cross was made. The following list alphabetically gives the symbols encountered in the selected tree and crossing system.

To illustrate, T-2-56 = the second P. tremuloides selected in 1956. XT-Ta-14-58 is the 14th cross made in 1958 and the cross involves a P. tremuloides female and a P. tremula male. XCa-G-18-70, S-1 = the first selected individual from the 18th cross in 1970 involving a P. canescens female and a P. grandidentata male.

A	= P. alba	M	= P. maximowiczii
An	= P. angustifolia	N	= P. nigra
B	= P. alba var. bolleana	O	= open pollinated
Ca	= P. canescens	S	= P. sieboldii
D	= P. deltoides	S-1, S-2, S-3, ...	= selected individuals
Da	= P. davidiana	T	= P. tremuloides
E	= P. euphratica	Ta	= P. tremula
G	= P. grandidentata	Tc	= P. balsamifera
Gla	= P. glandulosa	Tr	= P. trichocarpa
H	= hybrid	Ts	= P. tristis
I	= P. ilicifolia	X	= cross

GLOSSARY

Aspen. Refers to *P. davidiana*, *grandidentata*, *sieboldii*, *tremula*, and *tremuloides*. As used in this report, refers to species in the *Populus* section Leuce, which includes the aspens (subsection Trepidae Dode) and the true white poplars (subsection Albidae Dode) which includes *P. alba* and *P. canescens*. Hybrids of species within this section are also covered by this term. (See poplar.)

Auxins. A class of growth hormones causing cell enlargement.

Bh. Breast height (4.5 feet).

Bioassay. Determination of the relative effective strength of a substance by evaluating its effect on a test organism.

Bisexual. Having both functional male and female reproductive organs in the same flower, or in the case of *Populus*, a tree having both male and female flowers.

Canker. A necrotic area caused by fungi or bacteria. Hypoxylon cankers have a flat, sunken appearance that may or may not have callus ridges along the margin of the necrotic area.

Catkin. A scaly spike of usually unisexual flowers, as in *Betula* and *Populus*.

Chromosome. A microscopic, usually thread- or rodlike body carrying the units of inheritance (genes). The chromosomes are the primary constituents of the cell nucleus but are individually distinguishable only during nuclear division.

Chromosome number. The number or complement of chromosomes characteristic of a species. The number of sets must also be specified; thus, in *Pinus* the chromosome number may be expressed as "*n* equals 12" or as "2*n* equals 24," depending on whether sex cells or vegetative cells are observed.

Chromosome set. The chromosomes inherited as a unit from one parent. Most eggs or sperm carry only one set. A set usually includes one of each kind of chromosome characteristic of the species.

Clone. A group of plants derived from a single individual (ortet) by asexual reproduction. All members (ramets) of a clone have the same genotype and consequently tend to be uniform.

Cross. As used in the Aspen Genetics Program the term applies to progeny produced by mating trees of the same species (intraspecific) or of different species (interspecific).

Dbh. Diameter of the tree stem at breast height (4.5 feet).

Diploid. Having two sets of chromosomes in the nucleus - indicated by "2n." One-half of the chromosomes are contributed by the female parent, one-half by the male parent. Many higher organisms are diploid except for their sex cells and associated tissue.

Fertilization. Union of a haploid male sex cell with a haploid female egg cell to form a diploid zygote which develops into the normal tree.

Gene. The smallest unit that can be shown to be consistently associated with the occurrence of a specific genetic effect. The genes are ultramicroscopic and act as if linearly arranged at fixed places (loci) on the chromosomes. Each gene interacts with other genes and the environment to produce within the cell certain physiological effects that control the development of one or more characters of an individual.

Genotype. An individual's hereditary constitution, expressed or hidden, underlying one or more characters; the gene classification of this constitution expressed in a formula. The genotype is determined chiefly from breeding behavior and ancestry.

Haploid. Having the reduced chromosome number (n), i.e., having one set of chromosomes in the nucleus. This is normal in sex cells, which have only half of the number of sets occurring in diploid (2n) vegetative cells.

Heritability. A measure of the relative degree to which a character is influenced by heredity as compared to environment. The heritability (in the narrow sense) of a character in a population is the fraction of the total variation that is contributed by transmissible (additive) genetic differences, i.e., it is the ratio of genotypic variance to phenotypic variance. High heritability indicates that an individual's phenotype is indicative of its genotype and that differences in environment will cause little modification, i.e., that genetic control is high.

Heterosis. Hybrid vigor; the increased vigor of a hybrid as compared to the better parent. Heterosis is at a maximum in the F1 generation.

Heterozygosity. Presence in an organism of different members of the same allelic set, i.e., both the dominant and the recessive gene. For example, an Aa plant is heterozygous, whereas AA and aa plants are homozygous. A heterozygous individual characteristically does not breed true and is known as a hybrid with respect to the genes in question.

Homozygosity. Presence of identical alleles, either both dominant or both recessive, as for example AA or aa. A homozygous individual breeds true when mated with the same genotype for the character(s) in question.

Hybrid. As used in the Aspen Genetics Program the term applies to progeny produced as the result of mating trees of different species (interspecific).

Hybrid vigor. Same as heterosis.

Inbreeding. Intercrossing or selfing related organisms. This procedure, especially if carried out for a number of generations, exposes undesirable recessive characters and "fixes" desirable ones, i.e., renders them true-breeding.

Interspecific. Between species; e.g., interspecific hybridization is the production of offspring by cross-pollinating one species with another.

Intraspecific. Within a species; e.g., intraspecific hybridization is the production of offspring by cross-pollinating one individual of a species with pollen from another individual of the same species.

Mammatoxin. A term describing a poisonous substance produced by the fungus *Hypoxylon mammatum*.

Mutation. A sudden variation from the ancestral phenotype, due to gene or chromosome changes. If the cause can be demonstrated as a chromosome change, the mutation is preferably referred to by the specific phenomenon involved, e.g., a change in structure (aberration) or number (ploidy). Although mutations are infrequent, and usually recessive and harmful, they are the raw material of evolution and plant breeding.

Nucleus. The cell part made up chiefly of the chromosomes.

Ortet. The one plant from which members of a clone were originally derived.

Pathotoxin. A poisonous substance that is a product of metabolic activities.

Phenotype. (1) The demonstrable characteristic(s) of an organism; the product of the interaction of the genes of an organism with the environment. (2) Individual(s) described on the basis of demonstrable characteristics. Similar phenotypes do not necessarily breed alike.

Plantlet. A complete plant derived from a tissue culture system.

Ploidy. The chromosome situation with respect to number of sets, e.g., two sets (diploid), or variation from full sets (aneuploid).

Pollination. When pollen reaches the receptive catkin.

Polyploid. Having three or more times the haploid number of chromosome sets in its cells. A cell or organism having three sets (3*n*) is called triploid; four sets (4*n*) tetraploid.

Poplars. Refers to trees in the genus *Populus*. As used in this report, refers to species outside the section Leuce (see aspen).

Popple. A colloquialism which refers to native aspen, *P. tremuloides* and *P. grandidentata*.

Progeny test. Evaluation of the breeding value of parents by suitable comparisons among their offspring.

Putative. Suspected.

Ramet. An individual member of a clone.

Reciprocal cross. The repetition of a cross where the sexual function of the parents is reversed, i.e., female A x male B is the reciprocal of female B x male A.

Selection. Artificial selection is the propagation by man of organisms possessing desired characteristics. The aim generally is to improve the population or gain knowledge of its hereditary potentials. Natural selection is part of the evolutionary process resulting in the survival of the "fittest," i.e., of the best adapted individuals.

Sibs (siblings). Offspring, irrespective of sex, from the same parents but from separate fertilizations. Full sibs have both parents in common, half-sibs, only one in common.

Sprout. Vegetative shoot arising from the stump or roots. Root sprouts may also be designated as suckers.

Suckers. Vegetative shoots arising from subterranean roots or stems.

Tetraploid. See polyploid.

Tissue culture. A general term for organs, callus, or cells growing _in vitro_ on an artificial medium. Cultures can be started from a variety of plant parts which have cells capable of dividing.

Triploid. See polyploid.

Vegetative propagation. Propagation of a plant by asexual parts, as in tissue culture, budding, dividing, grafting, rooting, and air layering. Hereditary characteristics of the resulting clone (ramets) are identical with those of the original plant (ortet).